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(54) Title: PRODUCING INCREASED NUMBERS OF HEMATOPOIETIC CELLS BY ADMINISTERING INHIBITORS OF DIPEPTIDYL PEPTIDASE IV

(57) Abstract

Methods of producing increased numbers of hematopoietic cells by administering an inhibitor of dipeptidyl peptidase IV to such cells.

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PRODUCING INCREASED NUMBERS OF HEMATOPOIETIC CELLS BY ADMINISTERING INHIBITORS OF DIPEPTIDYL PEPTIDASE IV

Field of the Invention

The present invention relates to methods of producing increased numbers of hematopoietic cells through the administration of an inhibitor of dipeptidyl peptidase IV.

Background of the Invention

Dipeptidyl peptidase IV (DP-IV, also known as CD26) is a member of a class of proteolytic enzymes known as serine proteases. Found in humans and a variety of animal species, including yeasts, insects, frogs, pigs, and rats, DP-IV is expressed in mammals in the serum, the kidneys, the intestines, and some hematopoietic cells. The hematopoietic cells on which DP-IV has been identified include granulocytes, macrophages, thymocytes, and T-cells. The precise biological function of this enzyme in mammalian systems has not been well established. However, it has been shown to play a role in proteolyzing bioactive peptides in other animal systems.

The presence of DP-IV on the surface of some hematopoietic cells, and in particular on T-cells, has led to speculation that this enzyme might be involved in the functioning of the immune system. In one study, it was reported that administering an inhibitor of DP-IV to murine T-cells inhibits the antigen-induced proliferation of such cells, as well as the interleukin-2 (IL-2) production of those cells. (Flentke, George R., et al., Proc. Natl. Acad. Sci., USA 88, 1556-1559 (1991)).

It has also been suggested that DP-IV may play a role in regulating the activity of certain T-cell growth factors. Several cytokines, including IL-2, contain sites which may be susceptible to proteolysis by DP-IV. However, it has not been shown that these cytokines are actually substrates for DP-IV, nor has it been demonstrated that the biological activity of such cytokines would be altered even after proteolysis by DP-IV.

DP-IV is known to have a specificity for cleaving Xaa-Pro sequences from the N-terminus of a polypeptide, where Xaa

represents any amino acid used to build proteins in biological systems. Peptides comprising boronic acid analogs of amino acids are also known to be inhibitors of the serine proteases. The alpha-amino boronic acid Pro-boroPro has thus been found to be a potent inhibitor of DP-IV.

Summary of the Invention

In spite of prior art teachings that inhibitors of DP-IV suppress the proliferation of T-cells, the surprising discovery has been made that such inhibitors can in fact contribute to the production of increased numbers of hematopoietic cells (cells derived from multipotent hematopoietic stem cells).

This discovery will facilitate research involving hematopoietic cells. Inhibitors of DP-IV such as Pro-boroPro can be used to co-stimulate the proliferation of such cells, for example in combination with known cytokines. Such combinations stimulate cell growth more effectively than cytokines alone. Research into the immune system will be benefitted in particular, since many immune system functions are mediated by hematopoietic cells.

The present invention is also useful in treating a variety of disorders. Patients with AIDS, for example, suffer from depressed T-cell populations. These patients would therefore benefit from the administration of inhibitors of DP-IV, which can stimulate the proliferation of thymocytes such as T-cells.

The present invention will be of particular use in treating patients undergoing bone marrow transplants. By administering an inhibitor of DP-IV, the population of hematopoietic cells transplanted in such a procedure can be expanded in the donor before being transplanted, in vitro after being removed from the donor, and in the recipient after transplantation. The chances that the procedure will be a success are thereby increased by the use of inhibitors of DP-IV.

One method of producing increased numbers of hematopoietic cells is to remove such cells from a mammal and

administer to those cells an effective dose of an inhibitor of DP-IV, such as Pro-boroPro, along with other factors in vitro. This method can be used to stimulate the growth of cells used in laboratory cultures. Such cells can also be transplanted into another mammal of the same species for experimental or therapeutic purposes.

In a preferred embodiment, hematopoietic cells are removed from a mammal in need of increased numbers of hematopoietic cells, co-stimulated with an effective dose of an inhibitor of DP-IV and a growth factor such as a cytokine, and then reintroduced into the same mammal. In this way, the hematopoietic and/or immune system of the mammal is strengthened.

In another preferred embodiment, a mammal with a deficiency of hematopoietic cells is identified and an inhibitor of DP-IV, such as Pro-boroPro, is administered to that mammal directly. Preferably, the inhibitor of DP-IV is administered with an acceptable pharmaceutical carrier. Such an inhibitor can be administered orally, intravenously, intraperitoneally, intramuscularly, or in any other appropriate fashion.

Inhibitors of DP-IV are normally used in conjunction with proliferative agents or growth factors such as cytokines to co-stimulate the proliferation of hematopoietic cells. For example, IL-1, IL-2, IL-3, GM-CSF, or erythropoietin can be used along with inhibitors of DP-IV. When Pro-boroPro is applied to cell cultures in vitro, it has been found that such growth factors are required to stimulate cell proliferation. The use of a combination of a growth factor and an inhibitor of DP-IV has the benefit of reducing the dosage of each agent required to produce increased numbers of cells compared to using any of these agents alone. Thus, the side-effects produced by using these agents alone can be reduced or avoided.

Description of the Drawings

Figure 1 is a graph showing the results of an experiment testing the effect of Pro-boroPro on the number of thymocytes

in a rat thymus, where Pro-boroPro is administered to the thymuses of embryonic rats in their 17th day of gestation and to the thymuses of newborn rats.

Figure 2 is a graph showing the results of an experiment testing the effect of Pro-boroPro on the hematopoietic precursors of granulocyte and macrophage cells from the bone marrows of rats.

Detailed Description of the Invention

The methods of the present invention involve administering inhibitors of DP-IV to hematopoietic cells in order to produce increased numbers of such cells. It has been found that Pro-boroPro, a highly specific transition stage inhibitor of DP-IV, works well in the present methods. Any other inhibitor of DP-IV, however, is also within the scope of the present invention, including other amino-boronic acids with the formula Xaa-boroPro, where Xaa is any amino acid used in biological systems to build proteins. For example, the Xaa-boroPro compounds Lys-boroPro, Arg-boroPro, His-boroPro, Asp-boroPro, Glu-boroPro, Gly-boroPro, Asn-boroPro, Gln-boroPro, Cys-boroProd, Ser-boroPro, Thr-boroPro, Tyr-boroPro, Ala-boroPro, Val-boroPro, Leu-boroPro, Ileu-boroPro, Phe-boroPro, Met-boroPro, and Trp-boroPro are within the scope of the present invention. Other inhibitors of DP-IV, including other serine protease inhibitors, can be identified by one of skill in the art by screening such compounds, a matter of routine experimentation.

Pro-boroPro is relatively unstable at physiological pH, having a half-life of approximately 1.5 hours. However, it has been found that Pro-boroPro may be stored indefinitely at low pH. Preferably, Pro-boroPro is stored in solution at pH 4.0.

The hematopoietic cells used in the methods of the present invention include thymus-derived cells, macrophages, granulocytes, and other hematopoietic cells. As shown in Figure 1, thymocytes isolated from newborn and 17-day embryonic rat thymuses exhibit marked proliferation in response to treatment with Pro-boroPro. The thymuses of 17-

day embryonic rats contain primarily CD4-/CD8- (double negative, or DN) thymocytes, while the thymocytes in the thymuses of newborn rats contain a mixed population of DN cells, CD4+/CD8+ thymocytes, and mature CD4+/CD8- or CD8+/CD4- cells. The number of thymocytes recovered from both types of thymic lobes, when exposed to Pro-boroPro once every day for five days, increased compared to the number of thymocytes recovered from lobes not exposed to Pro-boroPro. The preferred dose of Pro-boroPro in these cultures is approximately 10^{-6} moles per liter in vitro, although Pro-boroPro in the range of 10^{-7} moles per liter to 10^{-6} moles per liter in vitro is efficacious.

Granulocyte and macrophage precursors also respond to the DP-IV inhibitor Pro-boroPro. Bone marrow cells from Buffalo rats were treated in vitro with Pro-boroPro. The results, as seen in Figure 2, show that the number of granulocyte and macrophage colonies increased in response to stimulation by Pro-boroPro and spleen supernatant, a source of growth factors. When treated once with Pro-boroPro, the preferred dose of Pro-boroPro for macrophage colonies is 10^{-6} moles per liter, although treatments in the range of 10^{-8} moles per liter to 10^{-7} moles per liter also stimulate proliferation. For granulocytes, 10^{-7} to 10^{-4} moles per liter of Pro-boroPro may be used, with a preferred range of 10^{-5} to 10^{-4} moles per liter.

Unlike the hematopoietic cells in thymic lobes, granulocytes and macrophages have been found to require, in addition to Pro-boroPro, a growth factor such as a cytokine for proliferation to occur. However, the combination of Pro-boroPro and another growth factor stimulates proliferation to a significantly greater extent compared to the use of that growth factor alone (see Figure 2).

A mammal in need of increased numbers of hematopoietic cells can be benefitted by the methods of the present invention. A person with AIDS, for example, is deficient in T-cells. Using the present methods, hematopoietic cells, such as T-cells, and bone marrow-derived precursors can be removed,

co-stimulated to proliferate by the administration of Pro-boroPro and other factors, and then reintroduced into the person from whom they were taken, thus strengthening that person's immune system and fostering hematopoiesis.

The removal of such cells can be accomplished by any of a number of techniques known to the art, depending on the cell type to be extracted. Once removed, the cells can be stimulated to proliferate according to the protocols of Examples 1 or 2. Cells grown up in this way can also be transplanted into another mammal of the same species in order to treat that mammal.

A mammal can also be treated for disease through the direct administration of inhibitors of DP-IV to that mammal. For example, Pro-boroPro or another suitable inhibitor can be injected directly into the bloodstream of a mammal in an appropriate pharmaceutical carrier, or can be administered orally.

A mammal suffering from a deficiency of hematopoietic cells can also be treated using the methods of the present invention. Such a mammal must first be identified, which can be accomplished through any of a number of methods known to the art. For example, a blood sample can be taken and then tested to determine whether the subject is deficient in T-cells or other hematopoietic cells carried in the blood. If the subject is found to be deficient in such cells, Pro-boroPro may be administered to that subject by any of the methods described above.

Detailed protocols for the above procedures, as well as other examples of how to practice the present invention, are given in the examples below.

Example 1

To investigate the effect of the DP-IV inhibitor Pro-boroPro on thymocytes, thymic lobes were removed from embryonic Buffalo rats (Charles River) on the 17th day of gestation and from newborn Buffalo rats. Single lobes were prepared by dissecting the two thymic lobes of each thymus. The lobes from the embryonic rats were placed onto a 0.8cm x

0.8cm x 0.8cm triangular, tissue culture quality polycarbonate membrane (Neuroprobe) and the membrane was placed over a culture medium comprising 45% RPMI 1640 (ABI), 45% Click's medium (Irvine Scientific Products), and 10% steroid free fetal calf serum (Hyclone) with a bicarbonate buffer. Since there may be variations in the degree of development of the various lobes due to differences in the individual rat embryos, the two lobes from each of the thymuses were grouped to reduce such variation. The same procedure was then followed with the thymic lobes from the newborn rats.

The lobes were cultured in a humidified incubator at 37°C with 5% CO_2 for 5 days. Pro-boroPro was administered at the initiation of the culture and once each day thereafter for the five days of culture. The Pro-boroPro was obtained from Dr. William Bachovchin at Tufts University (136 Harrison Avenue, Boston, MA 02111) and had a half life of about 1.5 hours at physiological pH. The lobes were made into a suspension of single cells at the end of the culture and the number of cells in each individual lobe was counted. The results, plotted in Figure 1, show that the stimulated thymus lobes treated with Pro-boroPro contained almost double the number of thymocytes in the untreated lobes. The optimal dose of Pro-boroPro was 10^{-6} moles per liter for both newborn and 17th-day gestational rat thymocytes under the conditions described above.

Example 2

To investigate the effect of DP-IV inhibitors on hematopoietic cells which are not thymus-derived, Pro-boroPro was administered to bone marrow cells containing granulocyte and macrophage precursor cells. Unfractionated bone marrow cells were removed from Buffalo rats (Charles River) and plated at a density of 5 x 10^4 cells per 2 ml medium (QBSF-56, Quality Biologicals, Inc.) containing 0.8% methylcellulose (Fluka), 3% conditioned spleen supernatant (as a source of growth factors), and 20% fetal calf serum (FCS, Hyclone) in 3.5 cm plastic petri dishes. The spleen cell supernatant was produced by stimulating Buffalo rat spleen cells (5 x 10^6/ml) with 5 micrograms/ml Con A (Sigma) in RPMI 1640 (ABI) medium

for 48 hours at 37°C. The Con A was removed by repeated passage of the supernatant over thyroglobulin-agarose columns (Sigma).

The bone marrow cells were cultured both with and without Pro-boroPro. When Pro-boroPro was administered to the cultures, only one treatment was given, and varying concentrations of Pro-boroPro were used. Colonies were grown at 37°C in a humidified incubator with 5% CO_2 and then counted on the eighth day of culture. The results are shown in Figure 2 in terms of the mean number of colonies in triplicate plates. As may be seen, the number of macrophage colonies increased by about 70% at the optimal dosage of Pro-boroPro for macrophages, while the number of granulocyte cells more than doubled at the optimal dosage for granulocytes. For macrophages, the optimal dose of Pro-boroPro under the above conditions is 10^{-6} moles per liter, although doses of 10^{-8} to 10^{-7} moles per liter are also effective in producing proliferation compared to the administration of no Pro-boroPro. For granulocytes, the preferred range of Pro-boroPro is 10^{-5} to 10^{-4} moles per liter administered under the conditions described above, although some increase in the number of colonies was observed at a dosage of as little as 10^{-7} moles per liter.

Example 3

The stimulated bone marrow-derived precursor cells produced in Example 2 above are separated from other bone marrow components through centrifugation at 1200 rpm for 5 minutes and then resuspended in 0.15 M saline solution. The major histocompatibility (MHC) antigens of these cells are cross-matched to the MHC antigens of rats which are possible transplantation recipients of such cells. A compatible recipient is selected, and the stimulated bone marrow derived cells are then transplanted into the selected rat through intravenous injection.

Example 4

Hematopoietic cells in the blood, such as T-cells, are removed from a human in need of increased numbers of such

cells. Such cells are separated from the blood and concentrated through centrifugation, and are then put in suspension in an appropriate buffer, such as 0.15 M saline.

An amount of Pro-boroPro which is effective to co-stimulate the proliferation of such cells in combination with a growth factor such as a cytokine is then administered to the cell suspension. In a preferred embodiment, enough Pro-boroPro is added to bring the concentration of Pro-boroPro in the suspension to between 10^{-8} and 10^{-4} moles per liter. Preferably, the concentration of Pro-boroPro is about 10^{-6} moles per liter. A cytokine such as IL-2 or GM-CSF is also added to the suspension at a concentration of about 1,000 BRMP units/ml. The suspension is treated once with Pro-boroPro and then cultured for up to eight days at 37 °C in a humidified incubator with 5% CO_2 to allow the cells to proliferate.

After proliferation, the cells thus stimulated are then reintroduced into the subject through the injection of the cells intravenously. The number of DP-IV-expressing cells in the subject is thereby increased compared to the number of such cells in the subject before the foregoing procedure.

Example 5

Thymocytes are removed from a child with a functional thymus. Pro-boroPro is then administered to these cells as in Example 1 along with a growth factor such as a cytokine in order to stimulate the proliferation of such cells. Following this treatment, the cells are suspended in a solution suitable for administration to the child and then reintroduced into the child through injection.

Example 6

Human subjects suffering from any disease or disease state, or from a deficiency of hematopoietic cells, for whom increased hematopoietic activity or increased T-cell production would be beneficial are first identified. Examples of such subjects include AIDS patients, patients undergoing chemotherapy treatment, or patients undergoing radiotherapy for hematological or other cancers. These subjects are then treated with an inhibitor of DP-IV. For example, they can be

treated through the intravenous administration of Pro-boroPro once daily in an appropriate pharmaceutical carrier at a dosage of about 1-10 mg/kg. Alternatively, Pro-boroPro can be administered orally once or several times daily in an appropriate pharmaceutical formulation at a dosage of 1-10 mg/kg per day. Cytokines can also be administered in conjunction with Pro-boroPro to increase the production of hematopoietic cells in a subject.

Example 7

Hematopoietic cells are directly stimulated to proliferate in a human subject through the administration of Pro-boroPro. Between 1 mg/kg and 10 mg/kg of Pro-boroPro is suspended in an appropriate volume of a pharmaceutically acceptable solution. This solution is then administered intravenously at least once daily in order to allow the production of increased numbers of such cells. An oral formulation of Pro-boroPro as described above can also be administered.

Example 8

An agent suspected of being an inhibitor of DP-IV is screened for its ability to inhibit that enzyme, as described in "Thymocyte Co-stimulating Antigen is CD26 (Dipeptidyl-peptidase IV): Co-stimulation of Granulocyte, Macrophage and T-lineage Cell Proliferation via CD26," J. Immunol., 149:367 (1992). DP-IV is first purified from bone marrow cells harvested by aspirating the femurs and tibias of rats with HBSS using an 18 gauge needle. Alternatively, DP-IV can be purified from the lamina propria of the small intestine, the kidney, or the liver. Many ways of purifying proteins from cells known to the art can be used. One such way is to homogenize the collected cells in lysis buffer (0.1 M Tris, pH 7.2, 0.15 M NaCl, 2% volume/volume Triton X-100 and 1.8 mg/ml iodoacetamide), incubate the mixture at 4°C for 30 minutes, and then centrifuge it at 400 g for 30 minutes at 4°C. The lysate is collected and diluted in 0.1 M Tris, pH 7.2, 0.15 M NaCl to a final concentration of 0.5% Triton X-100. The lysate and 20 ml ConA-Sepharose (supplied by Pharmacia, Inc.,

Piscataway, New Jersey) are then incubated overnight with shaking. Following incubation the ConA-Sepharose column is washed extensively with a solution of 0.1 M Tris, pH 7.2, 0.5% volume/volume Triton X-100, and 0.15 M NaCl at 4°C, and the material bound to the column is eluted with 5 mg/ml alpha-methyl mannoside in 0.5% Triton X-100 lysis buffer.

The eluted material in solution, which contains the DP-IV enzyme, can be used in a competition assay, as described below, by adding a substrate of DP-IV and a suspected DP-IV inhibitor to this solution and then detecting the rate of substrate cleavage. If it is desired to further purify the DP-IV from the material eluted from the Sepharose column, however, the eluted material can be be purified by any of a number of methods known to the art. One way to further purify the DP-IV in solution is to pass the solution over a Sepharose immunoaffinity column conjugated with an antibody specific for DP-IV. Obtaining such antibodies is known to those of skill in the art. After extensive washing of the column with 0.5% Triton X-100 lysis buffer, the antibody specific for DP-IV is eluted from the column with 0.2 M glycine, pH 2.0 containing 0.1% Triton X-100 in 5 ml fractions. The fractions are neutralized to pH 7.6 by the addition of 1 M Tris, pH 7.6. After concentrating the fractions, aliquots from each fraction are analyzed on a 7.5% SDS-PAGE gel. The gel is silver stained and analyzed to determine which fractions contained the DP-IV enzyme. The enzyme-containing fractions are then pooled, concentrated to 200 μl and made to 20% glycerol for storage at -70°C until needed.

Methods of screening inhibitors of DP-IV are known to the art, and any of these can be used in searching for inhibitors of DP-IV (see, e.g., "Diprotins A and B, Inhibitors of Dipeptidyl Aminopeptidase IV, Produced by Bacteria", J. Antibiotics, April, 1984, vol. 37, no. 4, pp. 422-425). In order to test a suspected inhibitor of DP-IV for inhibitory activity, any of a number of competition assays can be performed, as is known to those of skill in the art (see,

e.g., "Inhibition of dipeptidyl aminopeptidase IV (DP-IV) by Xaa-boroPro dipeptides and use of these inhibitors to examine the role of DP-IV in T-cell function", Proc. Natl. Acad. Sci. USA, February, 1991, vol. 88, pp. 1556-1559) various concentrations of the suspected DP-IV inhibitor (e.g., 0.0 mM, 0.01 mM, 0.02 mM, 0.04 mM, 0.1 mM, and 0.2 mM) are then added to a solution containing 50 μM sodium Hepes (pH 7.8), 10 μM Ala-Pro-4-nitroanilide (a substrate of DP-IV), 6 milliunits of the DP-IV collected as described above, and 2% (volume/volume) dimethylformamide brought to a total volume of 1.0 ml with water. These solutions are incubated at approximately 25°C and are sampled approximately every minute for about 10 minutes to monitor the amount of substrate (Ala-Pro-4-nitroanilide) cleaved by DP-IV. When Ala-Pro-4-nitroanalide is cleaved by DP-IV, para-nitroanaline is formed. This compound appears yellow in solution, so the amount of Ala-Pro-4-nitroanilide cleaved can be measured by measuring the color change of the solution with a spectrometer at approximately 410 nanometers.

The degree of inhibition of substrate cleavage by the suspected DP-IV inhibitor is then determined by comparing the rate of substrate cleavage by DP-IV in the presence of the suspected inhibitor to the rate of substrate cleavage when no inhibitor is present. If the rates of substrate cleavage by DP-IV in the solutions containing the suspected DP-IV inhibitor are not statistically different from the rate of cleavage in the solution containing no inhibitor, then the screened compound is not a DP-IV inhibitor.

The foregoing embodiments of the present invention are illustrative only and do not limit the present invention. Suitable inhibitors of DP-IV and suitable hematopoietic cells not explicitly mentioned herein may be easily identified through routine experimentation by those skilled in the art.

WHAT I CLAIM IS:

1. A method of producing increased numbers of hematopoietic cells by administering an inhibitor of dipeptidyl peptidase IV to such cells, comprising the steps of:

removing hematopoietic cells from a mammal; and

administering an effective amount of an inhibitor of dipeptidyl peptidase IV to said cells.

2. The method of Claim 1, including the step of transplanting said cells into another mammal of the same species.

3. The method of Claim 1, wherein said inhibitor is Xaa-boroPro, Xaa being any amino acid used to build proteins in biological systems.

4. The method of Claim 3, wherein said inhibitor is Pro-boroPro.

5. The method of Claim 1 above, wherein a growth factor is administered with the inhibitor of DP-IV.

6. The method of Claim 5, wherein said growth factor is selected from the group consisting of IL-1, IL-2, IL-3, Con A, erythropoietin, and GM-CSF.

7. A method of treating a mammal in need of increased numbers of hematopoietic cells, comprising the steps of:

removing hematopoietic cells from a mammal;

administering an effective amount of an inhibitor of dipeptidyl peptidase IV to said cells; and

reintroducing said cells into said mammal.

8. The method of Claim 7, wherein said inhibitor is Xaa-boroPro, Xaa being any amino acid used to build proteins in biological systems.

9. The method of Claim 8, wherein said inhibitor is Pro-boroPro.

10. The method of Claim 7, wherein a growth factor is administered with the inhibitor of DP-IV.

11. The method of Claim 10, wherein said growth factor is selected from the group consisting of IL-1, IL-2, IL-3, Con A, erythropoietin, and GM-CSF.

12. The method of Claim 7, wherein said mammal is suffering from an immunodeficiency disease such as AIDS.

13. A method of treating a mammal having a deficiency of hematopoietic cells, comprising the steps of:

identifying a mammal with a deficiency of hematopoietic cells; and

administering to said mammal an effective dose of an inhibitor of dipeptidyl peptidase IV.

14. The method of Claim 13, wherein said inhibitor is Xaa-boroPro, Xaa being any amino acid used to build proteins in biological systems.

15. The method of Claim 14, wherein said inhibitor is Pro-boroPro.

16. The method of Claim 13, wherein said inhibitor is carried in a pharmaceutically acceptable carrier.

17. The method of Claim 13, wherein a growth factor is administered with said inhibitor.

18. The method of Claim 17, wherein said growth factor is selected from the group consisting of IL-1, IL-2, IL-3, Con A, erythropoietin, and GM-CSF.

19. The method of Claim 13, wherein said administration step is selected from the group consisting of intravenous injection, intraperitoneal injection, and intramuscular injection.

20. A method of producing increased numbers of hematopoietic cells in a mammal, comprising the step of administering to said mammal an effective dose of an inhibitor of dipeptidyl peptidase IV.

21. The method of Claim 20, wherein said inhibitor is Xaa-boroPro, Xaa being any amino acid used to build proteins in biological systems.

22. The method of Claim 20, wherein said inhibitor is Pro-boroPro.

23. The method of Claim 20, wherein said inhibitor is carried in a pharmaceutically acceptable carrier.

24. The method of Claim 20, wherein a growth factor is administered with said inhibitor.

25. The method of Claim 24, wherein said growth factor is selected from the group consisting of IL-1, IL-2, IL-3, Con A, erythropoietin, and GM-CSF.

26. The method of Claim 20, wherein said administration step is selected from the group consisting of intravenous injection, intraperitoneal injection, and intramuscular injection.

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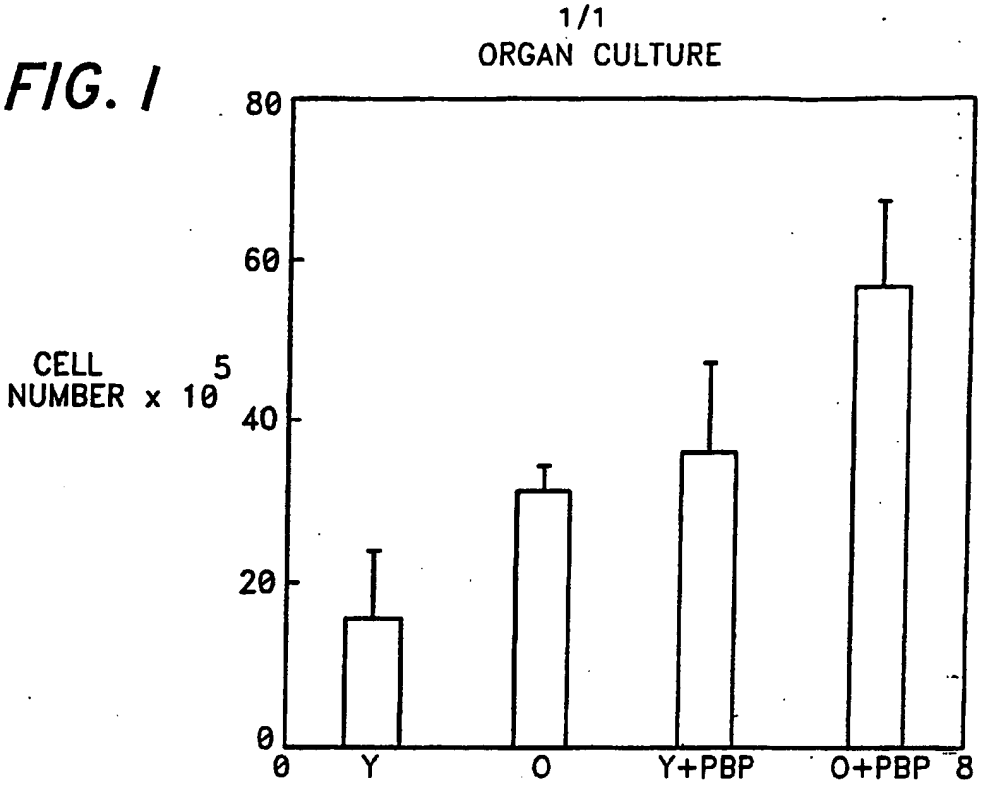
FIG. 1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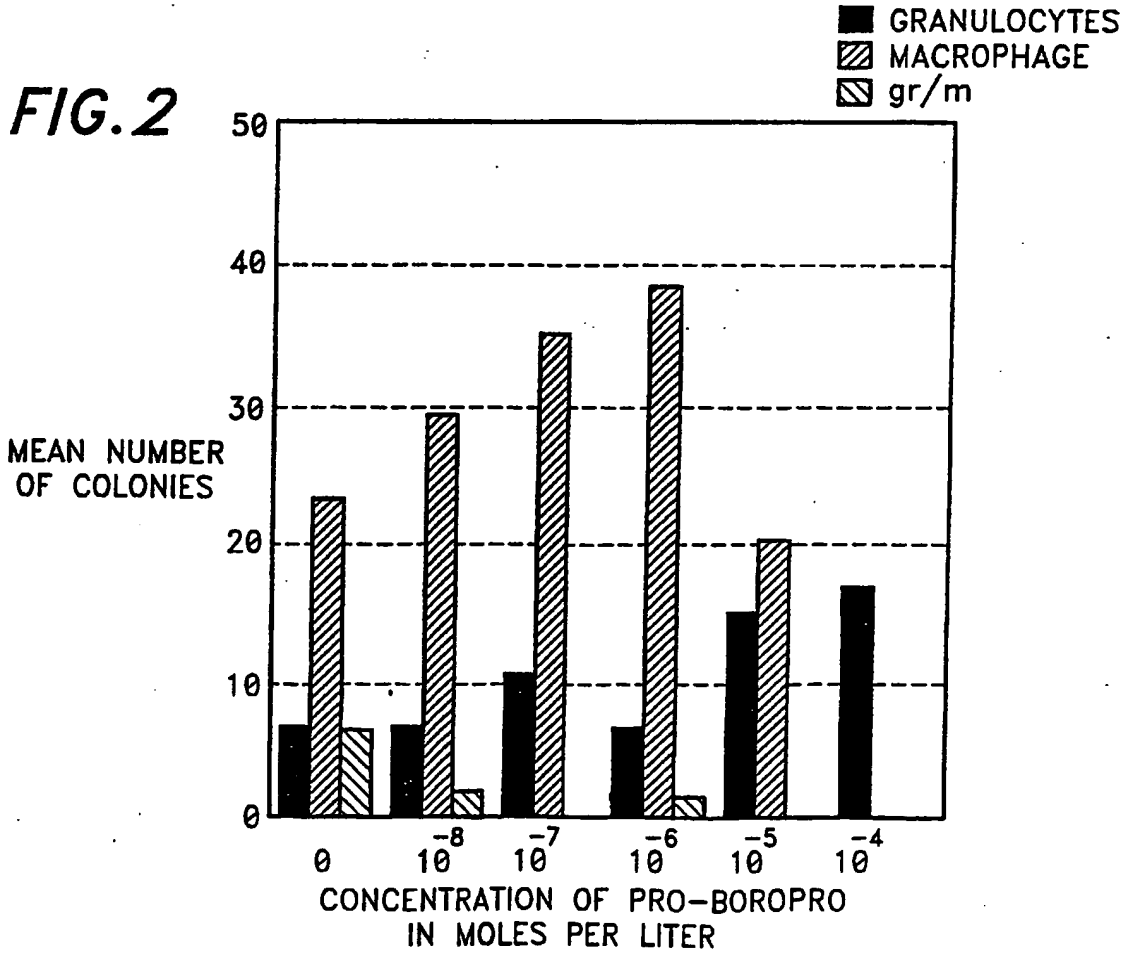
O
Y+PBP
O+PBP
8

FIG. 2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gr/m
MEAN NUMBER OF COLONIES
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10
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0
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10^-7
10^-6
10^-5
10^-4
CONCENTRATION OF PRO-BOROPRO IN MOLES PER LITER

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US93/07173

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A01N 01/02; A61K 37/00, 37/64, 31/69, 33/22
US CL :435/2; 424/657; 514/2, 64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/2; 424/657; 514/2, 64

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Medline, Biosis, Chem Abstracts, Derwent WPI, search terms: CD26, dipeptidyl peptidase, bone marrow, macrophage, author names, peptidase, bestatin, hematopoiesis, colony formation

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proceedings of the National Academy of Science USA, Volume 88, issued February 1991, G.R. Flentke et al.,"Inhibition of dipeptidyl aminopeptidase IV (DP-IV) by Xaa-boro-Pro dipeptides and use of these inhibitors to examine the role of DP-IV in T-cell function", pages 1556-1559, see entire document.	1-26
Y	The Journal of Antibiotics, Volume 37, No. 4, issued April 1984, H. Umezawa et al.,"Diprotons A and B, inhibitors of dipeptidyl aminopeptidase IV, produced by bacteria", pages 422-425, see entire document.	1-26

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
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Date of the actual completion of the international search 01 SEPTEMBER 1993	Date of mailing of the international search report 07 SEP 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer DAVID LACEY Telephone No. (703) 308 0196

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INTERNATIONAL SEARCH REPORT

International Application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	The Journal of Antibiotics, Volume 40, issued June 1987, K. Nemoto,"Enhancement of colony Formation of mouse bone marrow cells by ubenimex", pages 894-898, see entire document.	1-26
Y	Cancer Immunology Immunotherapy, Volume 29, Issued April 1989, F. Abe et al.,"Chemoimmunotherapy with cyclophosphamide and bestatin in experimental metastasis in mice", pages 231-236, see entire document.	1-26